**Presentation 8 – Glenn Ritchie**

## Possible Role of Hydrocarbon Fuel Exposures on Development of Gulf War Illnesses

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## Qualifications

- 10 years-Assistant Director of the Navy Neurobehavioral Effects Laboratory, Wright-Patterson AFB, OH.
- First animal study of Gulf War / jet fuel "synergism".
- 2 major reviews of hydrocarbon fuel toxicity.
- A number of "fuel" neurobehavioral effects publications.
- Neurobehavioral research in USAF 7-Base human study
- Former Navy "expert" for hydrocarbon fuel toxicity.
- Invited presentations to JANNEF and International Jet Fuel Toxicity Conferences.
- Member, USAF JP-8 Research Consortium.
- Navy expert for the Fallon "Angels" leukemia cluster.
- Involved in the Sierra Vista, AZ cluster investigation.

## Presentation Objectives

1. Define the different hydrocarbons present in the Persian Gulf theater.
2. Discuss warfighter routes of exposure and hydrocarbon fuel exposure scenarios.
3. Briefly discuss direct health effects of repeated fuel exposures.
4. Provide data on hydrocarbon health effects additivity & synergism with other PGW toxicants.

## Overall Objective

**To provide evidence that repeated hydrocarbon exposures can (additively or synergistically) increase the effects of exposure to other "military" theater toxicants, and possibly contribute to induction of Persian Gulf illnesses.**

## Caveats

1. Clearly, human effects of hydrocarbon exposures appear limited to "minor" CNS, dermal, lung, blood, reproductive, kidney, liver and immune system deficits.
2. Millions exposed repeatedly to hydrocarbon fuels do not become seriously ill.

– but –

**Exposure to hydrocarbon fuels and "unique" chemicals and environments may result in illnesses unpredicted by exposure to other Persian Gulf toxicants**

# Hydrocarbons Present in the Persian Gulf

## Hydrocarbons Present in the Persian Gulf

- **JP-8 (USAF & US Army) – JP8 (100)??**
- **JP-5 (US Navy)**
- **JP-4 (Turkey, Saudi Arabia & other Allies)**
- **Kerosene**
- **Diesel and Marine Diesel**
- **Limited AVGAS & Gasoline**
- **Numerous Lubricants & Solvents**
- **Large Quantities of Fuel Additives**
- **Jet-A, AVGAS, gasoline**
- **Jet Oils (tricresyl phosphate)**

## What is JP-8?

- 220 hydrocarbons; 2000+ isomeric forms.
- Complex mixtures of aliphatic, aromatic and substituted naphthalene hydrocarbons (C4-C22).
- Benzene, methylbenzenes, ethylbenzenes, cyclohexylbenzenes.
- Xylenes.
- Toluene.
- Naphthalenes.
- Known and proprietary fuel additives that may contain BHT, DIEGME (diethylene monomethyl ether), xylenes, toluene and benzene.

## Predicting the "Toxicity" of Hydrocarbon Fuels

- Each hydrocarbon fuel has a unique "hydrocarbon cut" and additive package.
- Fuels produced by different refineries are substantially different in chemical content.
- Each new iteration of jet fuel is less volatile than the previous version, increasing dermal exposure potential.
- Volatile hydrocarbons removed from fuels to reduce toxicity are replaced with proprietary additives that may contain similar toxicants.
- For example, Kuwaiti crude oil is very different from crude oil in the US.

## Routes of Exposure and Hydrocarbon Fuel Exposure Scenarios

## Routes of Exposure

- **Inhalation of fuel vapor.**
- **Inhalation of fuel aerosol (vapor/aerosol).**
- **Inhalation of sand aerosols.**
- **Inhalation of combusted hydrocarbons.**
- **Direct dermal exposure to neat fuels.**
- **"Second-hand" exposure to contaminated clothing (family).**
- **Hydrocarbons mixed in drinking or shower water.**
- **Hydrocarbons mixed in food.**

## Exposure Scenarios

**Neat fuels – conventional scenarios**

- Fuel transportation (truck, aircraft and pipeline).
- Fuel handling.
- Fuel storage .
- Fueling of aircraft, land craft, equipment, heaters.
- Fuel related to vehicle/aircraft operation.

"Presence at any location where fuels were used."

## Exposure Scenarios

**Neat Fuels – unconventional scenarios**

- Cold start-up of aircraft (up to 10% raw fuel released).
- Numerous fuel spills & leaks.
- Equipment, munitions and vehicle cleaning with fuels.
- Use in vented / unvented tent heaters.
- Vehicle-delivered sand suppression using fuels.
- Aircraft-delivered sand suppression using fuels.
- Kuwaiti oil fires (?% uncombusted oil).
- Aircraft fuel tank upper atmosphere "dumping".
- Use of JP-8 in cooling systems.
- Smoke screen (obscurant) generation.
- "Apple Jelly" containing LPS (C8-C10 fractions).

## Hydrocarbon Combustion Byproducts

- Neat fuel (up to 10%)
- Up to 30 polycylic aromatic hydrocarbons (PAHs), many of which are known carcinogens.
- Carbon monoxide
- NOx
- Formaldehyde
- SOx
- Extensive respirable particulates

## Direct Health Effects of Repeated Hydrocarbon Exposures

## CNS Effects of Fuel Exposures

- Severe psychiatric symptoms; neurasthenia; polyneuropathy; shortened attention span; EEG alteration; reduced auditory evoked cortical potentials (Knave and associates, 1976-1980).
- Increased human postural sway (Smith et al., 1997).
- Human exposed chronically (National Guard) exhibited significantly poorer performance of 20/47 NeuroCog Battery measures (Mitchell, Kay and Risby, 2004; Anger et al.).
- Jet fuelers were impaired on acquisition of classically conditioned eyeblink response (Bekkedal, Rossi & Ritchie).
- Hearing impairment (synergistic fuel x noise) [Kaufman et al., 2005]. Gene expression changes related to CNS neurotransmitter signaling pathways (Lin, Ritchie et al., 2004).

## CNS Effects of Fuel Exposure

- Rats were exposed by whole body inhalation to 1000 mg/kg (H), 500 mg/kg (L) or 0 mg/kg (C) JP-8 vapor for 6 h/d, 5 d/w, for 6 w. (Ritchie et al., 2001).
- After 65-d rest, rats (n = 15) were trained on simple, moderate difficulty, and complex operant tasks.
- All learned simple tasks equally well; low-dose learned moderate difficulty tasks slightly better than high dose; the high dose group was greatly impaired on learning the complex task (IRA, incremental repeated acquisition) compared to controls or the low dose group.

## Dermal Toxicity

- One of the major complaints of military fuel workers is chronic dermatitis (increased ROS). (Riviere, McDougal).
- Dermal absorption is related to carbon chain length (mild irritation to skin cancer) and exposure duration.
- JP-8 induces dermal irritancy: erythema, epidermal edema, increased epidermal thickness, subcorneal microabcesses, and dermal microlesions/lesions.
- Induction of proinflammatory cytokines (IL-1-alpha, TNF-alpha, IL-8).
- Lipid extraction from the stratum corneum.
- Dermal exposure to JP-8 resulted in significant changes in protein expression in 35/929 dermal proteins surveyed (Witzmann et al., 2005).

## Immune & Blood Effects

- Keil et al. (2004) (mice, 14 days, JP-8 gavage)-reduced hematocrit, hemoglobin concentration, and red blood cell count; increased liver mass; decreased thymic cellularity; alterations in splenic CD4/8 subpopulations with high dose gavage.
- Reduced thymus weight and immune cell populations in thymus; decreased immune function as identified by mitogenesis assays (Harris et al., 2001 - dermal application).
- Blood and bone marrow genotoxicity (micronuclei) [Vijayalaxmi et al., 2004 – vapor / aerosol exposure).
- (Respiratory) Natural Killer (NK) cell function was nearly eliminated, and lymphokine-activated killer cell activity was suppressed (Harris et al., 2000).

## Immune & Blood Effects

- Five minimal applications, or one large dermal application of JP-8 induced immunosuppression in mice (Ullrich, 1999).
- Contact hypersensitivity to a bacterial antigen was significantly suppressed.
- The ability of splenic T lymphocytes to proliferate was suppressed.
- IL-10, a potent immunosuppresssive cytokine, was found in serum
- JP-8 (100) > JP-8 > JP-4 in inducing genotoxicity in peripheral lymphocytes (Jackman et al., 2002 – cell cultures).

## Pulmonary Toxicity

- Increased lung compliance (Pfaff et al., 1995).
- Increased lung epithelial permeability (Hays et al., 1995).
- Decreased BALF concentrations of Substance P (Witten et al.).
- Increased protein levels in BALF (Robledo et al., 2000).
- Secretion if IL-1-beta, IL-6, Tumor Necrosis Factor (TNF)-alpha.
- Apoptosis in lung epithelial cells (Stoica et al., 2001).
- Dose-related protein up regulation (30 at 2500 mg/m3) and down regulation (135 at 2500 mg/m3) [Drake, Witzmann et al., 2003].

## Additional Toxicity

- Minimal evidence of hepatotoxicity (Witzmann et al.)
- Limited effects on male and female reproductive systems (LeMasters and Colleagues; Witzmann et al.).
- Renal toxicity that seems limited to rodent species (Mattie and Colleagues; Witzmann et al.)

## Additivity and Synergism Data Related to Hydrocarbon Exposures

## Major Exposure Co-Factors

- Physiological / psychological stress
- PB and/or atropine
- DEET, Permethrin & 63 other pesticide treatments
- Tungsten, DU & lead munitions
- Aerosolized DU and W
- Desert environment – heat/cold
- Sand aerosols
- Numerous hydrocarbon solvents, cleansers
- Low-level sarin (sulfur mustard?) exposures
- Anti-war gas treatments (PB and atropine)
- Population mixing
- Known and unidentified vaccinations
- Endogenous parasites and infections
- Sewage and water quality

## Desert Aerosols

- Persian Gulf sand varies by location, and contains respirable and non-respirable particles.
- Frequent winds and vehicle movements result in "sand aerosols"
- Toxicants in the sand can become attached to respirable and non-respirable particles that enter the lungs
  - Dumped and spilled fuels
  - Depleted tungsten, DU and lead munitions
  - Animal (bird) wastes
  - Human wastes

## Persian Gulf Study (1994)

- **Male S-D rats were exposed for 6/hr day for 14 days to:**
  - JP-4 vapor (1000 mg/kg)
  - Unpredicted footshock stress
  - DEET (shaved skin/acetone) & PB (corn oil)
- **Half were rested 14 days, then tested**
- **Half were rested 60 days, then tested**
- **Eight neurobehavioral tests (all animals)**
- **Neurotransmitter levels in 4 brain areas**

## Persian Gulf Study (1994)

- **Exposure scenarios:**
  - **Control (C)**: Room Air, Corn Oil, Acetone
  - **Vapor (V):** JP-4 vapor
  - **Stress (S)**: Unpredicted footshock
  - **PB & DEET (PB&D)**: PB in corn oil (gavage) and DEET in acetone (dermal to shaved skin)
  - **Treatment Groups:** C, V only, S only, PB&D only, V/S, V/PB&D, S/PB&D, V/S/PB&D

## Persian Gulf Study Results

- **JP-4/S decreased grip strength (14-d); stress increased grip (60-d).**

- **JP-4 increased ARAS (14-d); stress/PB&D increased ARAS (60-d).**
- **Stress, JP-4/Stress and Stress/PB&D, decreased acoustic startle response (14-d).**

## Persian Gulf Study Results

- **JP-4 or stress/PB&D decreased prepulse inhibition (60-d).**

- **JP-4 decreased total locomotor activity (60-d).**

- **JP-4, stress, or stress/PB&D decreased tail flick latency (60-d).**

## CNS Neurotransmitters

**CNS neurotransmitter levels are modulated by JP-4 or JP-5 inhalation exposures (measured as long as 80+ days post-exposure) (Nordholm et al, 1999; Ritchie et al., 2001):**

- **Depleted dopamine (DA).**
- **Elevated DA metabolite dihydroxphenylacetic acid (DOPAC).**
- **Elevated serotonin (5-HT).**
- **Increased 5-HT metabolite homovanillic acid (HVA).**
- **Increased 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA).**

## Fuel Synergism

- A number of human metabolic interactions have been identified among chlorpyrifos, carbaryl, DEET, permethrin, PB, solvent and jet fuel exposures.
- **Permethrin** has been shown inhibit the metabolism of DEET (cytochrome P450) and carbaryl (Hodgson and Rose, 2005).
- **JP-8** has been shown to inhibit CYP 1A2 and 2B6 metabolism of DEET (Edwards et al., 2005).

## Synergism Studies

- **Riviere et al., 2002.**
- **Isolated porine skin flap (soaked fabric occlusion).**
- **Low level sulfur mustard, JP-8 + DEET on transdermal absorption of permethrin.**
- **Normally DEET or sulfur mustard inhibits permethrin dermal absorption.**
- **JP-8 increased permethrin percutaneous absorption (2x) and skin penetration (3x).**

## Antioxidant Effects

- JP-8 induces generation of reactive oxygen species (ROS).
- JP-8 significantly depletes intracellular glutathione stimulating hormone (GSH) (Smulson et al, 2002).
- Aerosolized JP-8 reduced glutathione-*S*-transferase (GST) in the retina (McGuire et al., 2000).

## Fuel Synergism

- Fuel exposure radically alters dermal and pulmonary system permeability to other potential toxicants.
- Fuel exposure suppresses the immune system.
- Hydrocarbon exposures suppress normal xenobiotic metabolism systems.
- Fuels may increase generation of Reactive Oxygen Species.
- Fuel may interact synergistically with heavy metals (i.e., W, Arsenic, DU) to induce blood-related cancers.

## The Fallon "Angels"

- **Fallon, NV (24,000 residents & military)**
- **Desert environment**
- **Much JP-8 use, leaking, dumping**
- **Top Gun relocated to Fallon NAS by 1998**
- **40,000 sorties/year**
- **Lead, DU and W munitions**
- **Highest arsenic (in water) in the world**
- **Tungsten mining/ smelting (in water, air, trees)**
- **1963 underground nuclear test**
- **Population mixing**

## Sierra Vista Cluster

- **Sierra Vista, AZ (28,000)**
- **Desert environment**
- **Ft. Huachuca (unmanned drones)**
- **Numerous "touch-downs"/year**
- **JP-8 / AVGas / Combustion Byproducts**
- **High arsenic (in air)**
- **Tungsten in water, air, trees**
- **Mexican Garbage Fires – Local Wildfires**
- **Mexican Cu Smelting**

## The Fallon "Angels" and Sierra Vista Equation

**Desert + Military Base + High Heavy Metals**
**=**
**As many as 17 cases of Acute Lymphocytic Leukemia (ALL) and Unexplained High Rates of Adult Cancers (cluster should occur by chance in US once every 22,000 years)**

## The Persian Gulf

- **Desert environment**
- **Military "bases"**
- **Extensive exposure to hydrocarbons**
- **Lead, DU, W munitions**
- **High physical/psychological stress**
- **Population mixing**

## Summary

- ❑ Warfighters in the Persian Gulf were/are repeated exposed, through multiple routes, to a wide diversity of hydrocarbons, as well as to numerous other toxicants.
- ❑ Repeated exposure to hydrocarbons is known to induce direct health effects in at least the CNS, skin, lungs, immune system, and blood.
- ❑ JP-8 can increase dermal and pulmonary absorption of xenobiotics, reduce metabolism of xenobiotics, reduce immune function, and increase levels of ROS.

## Summary

**It appears logical to assume that concurrent exposure to hydrocarbon fuels and other diverse "military theater" toxicants could induce health effects in at least susceptible individuals that might not otherwise occur.**

## Research Plan

- **Rats and/or monkeys**
- **Whole body exposures**
- **6 hr/day for 30 days**
- **Permethrin x DEET x JP-8 x Stress x Sand x Heat**
  - –Permethrin at normal human dermal dose
  - –DEET at normal human dermal dose
  - –Stress = unpredicted foot shock
  - –JP-8 vapor/aerosol mixture & dermal exposure
  - –Sand aerosol
  - –100 °F